"Express Mail" mailing label No. EV 346846807 US

Date of Deposit January 16, 2004

UNITED STATES PATENT APPLICATION

OF

Zongcen Charles Xie
Jeffrey A. Pierce
Carol R. Stivers

FOR

METHODS AND SYSTEMS FOR POINT OF CARE BODILY FLUID ANALYSIS

Attorney Docket No. 033819-071
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

METHODS AND SYSTEMS FOR POINT OF CARE BODILY FLUID ANALYSIS

RELATED APPLICATIONS

The subject matter of this application is related to diagnostic devices, systems and chemistry, and in particular is related to the disposable single-use digital electronic instruments that are entirely self-contained, including all chemistry reagents, as disclosed in U.S. application Serial No. 08/455,236 entitled "Novel Disposable Electronic Assay Device" filed May 31, 1995 by Michael P. Allen and now U.S. Patent 5,580,794; U.S. application Serial No. 08/657,894 entitled "Electronic Assay Device and Method" filed June 7, 1996 by Michael P. Allen, Joel M. Blatt, and Joseph T. Widunas and now U.S. Patent No. 5,837,546; U.S. application Serial No. 08/564,441 entitled "Device For Blood Separation in a Diagnostic Device" filed November 29, 1995 by Joel M. Blatt, Wilma M. Mangan, Paul J. Patel and Michael P. Allen and now U.S. Patent 5,981,294; U.S. application Serial No. 08/645,453 entitled "Method and Device Producing a Predetermined Distribution of Detectable Change in Assays" filed May 13, 1996 by Joel M. Blatt, Michael P. Allen and Paul J. Patel, now U.S. Patent 5,968,839 and U.S. application Serial No. 08/703,479 filed August 27, 1996 by Joel M. Blatt, Wilma M. Mangan, Paul J. Patel and Victor A. Manneh, now U.S. Patent 5,945,345. The above patents have the same assignee as the present invention and this application, and are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to diagnostic devices and systems used for qualitative and quantitative determination of analytes in bodily fluids, such as blood and urine.

BACKGROUND OF THE INVENTION

There has been a proliferation of analytical and diagnostic devices known as "point of care" devices. These devices are used professionally in clinics, doctors' offices, and hospitals, and are used by individual consumers for glucose, glycated hemoglobin, pregnancy and other tests. These devices are well known in the art and typically include a reagent pad in the form of a strip or built into a device for receiving a bodily fluid sample, and a meter or indicator

device for giving the user a readout of the analysis results, such as glucose level, or a color indication of a condition present or not present, such as, pregnancy. In some cases the device is part of a test kit or system, which also includes a sample preparation portion, such as a sample dilution vial and solution.

Some of the point of care devices and kits have limited shelf life, including some devices and kits that are best kept under refrigeration, e.g., below about 10° C, in order to provide useful shelf life, particularly in the consumer or individual use market. For example, the METRIKA® A1c test kit (available from Metrika, Inc., Sunnyvale, California) for glycated hemoglobin testing is recommended for storage under refrigeration in order to provide a one year shelf life. Without refrigeration, the Metrika A1c test kit is recommended for use within one month.

Thus, there is a need for improvement in point of care diagnostic devices and test kits to provide extended shelf life and in particular for extended shelf life without refrigeration.

SUMMARY OF THE INVENTION

This invention provides a system for quantitative measurement of percent glycated hemoglobin as hemoglobin A1c in whole blood comprising a blood dilution solution and a device adapted for receiving at least a portion of diluted blood solution, for contacting the blood solution with a dry reagent system, for detecting a change in the reagent system and for providing an indication of the analytical result to the user, wherein the blood dilution solution comprises a first surfactant for hemolysis and a second surfactant for stability. In a particular aspect. this invention provides said system wherein the first surfactant is a zwitterionic surfactant and the second surfactant is a nonionic surfactant.

In another aspect, this invention provides a composition for dilution of a bodily fluid for analysis comprising a first surfactant for modification of an analyte in the fluid and a second surfactant for stability. The composition further comprises a bodily fluid in mixture

with a first surfactant for modification of an analyte in the fluid and a second surfactant for stability, and in particular wherein the first surfactant is a zwitterionic surfactant and the second surfactant is a nonionic surfactant.

In another aspect, this invention provides a method of preparing a whole blood sample for analysis comprising diluting the blood sample with a solution comprising a first surfactant for hemolysis and a second surfactant for stability. The diluted blood sample is contacted with a dry immunoassay reagent system. In a particular aspect this invention provides said method wherein first surfactant is a zwitterionic surfactant and the second surfactant is a nonionic surfactant.

In another aspect, this invention provides a system for detection of an analyte in a liquid sample comprising a sample dilution solution and a device adapted for receiving at least a portion of diluted sample solution, for contacting the sample solution with a dry reagent system, for detecting a change in the reagent system and for providing an indication of the analytical result to the user, and wherein the sample dilution solution comprises a first surfactant for modification of the analyte and a second surfactant for stability. In a particular aspect, this invention provides said system wherein first surfactant is a zwitterionic surfactant and the second surfactant is a nonionic or an ionic surfactant. In another particular aspect, this invention provides said system wherein the first surfactant is a nonionic surfactant and the second surfactant is a zwitterionic surfactant or an ionic surfactant. In another particular aspect, this invention provides said system wherein the first surfactant is an ionic surfactant and the second surfactant is a zwitterionic surfactant or a nonionic surfactant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 and 2 show the effect of a nonionic surfactant on stability of a test unit.

Figs. 3 and 4 show the stability of the test kit using the sample treatment buffer without the nonionic surfactant added.

Figs. 5 - 8 show the stability of the test kit using the sample treatment buffer with the nonionic surfactant present in different amounts.

DESCRIPTION OF THE INVENTION

This invention provides an improvement in stability and shelf life for a variety of diagnostic devices, systems and kits. It is particularly useful in those devices and systems that employ a dry reagent test site and is preferred for use in such devices and systems employ a dry reagent system, particularly immunoassay reagent systems, which are commonly used in such devices and systems. This invention is implemented by providing a novel dilution solution for sample preparation prior to analysis. Conventional dilution solutions for sample preparation for dry reagent systems contain a single surfactant or a single type of surfactant for modification or preparation of the analyte in the sample fluid to provide compatibility and/or reactivity with the reagent system in the test device or test strip. It has now been found that when, in addition to the surfactant for modification or preparation of the analyte, a second surfactant is included, the dilution solution provides for increased stability and increased shelf life of the system as a whole. Thus, this invention includes modification of the sample dilution solution, which in turn provides increased stability and extended shelf life of the whole system comprising the dry immunoassay reagent system. As a result, the entire kit comprising the dilution solution, the dry reagent system and the reading device can exhibit extended shelf life at room temperature, without the need for low temperature storage to obtain the extended shelf life.

Disclosure and description of the invention will be in the context of blood analysis, particularly glycated hemoglobin (%HbA1c) testing systems known in the art. However, it will be apparent to one skilled in the art that this invention can be adapted to other systems to increase system stability and shelf life, particularly those systems that employ dry latex particle type reagent systems. The problem solved by the improvement of this invention is that over time, certain diagnostic and test systems degrade in accuracy to the point where they are unreliable and unusable. Different diagnostic systems have different stability and shelf life characteristics. It has been found that in a hemoglobin A1c test system, incorporating a nonionic surfactant as stabilizer with the conventional zwitterionic surfactant as a hemolysis agent, provides a system that substantially improves shelf life at room temperature. This invention is expected to enable reasonable shelf life with the elimination of the refrigeration requirement for such test systems.

The dry reagent systems for which this invention is useful in extending shelf life and stability of the analysis system or kit are those which comprise a binding pair assay in the form of particulate labels. Preferred systems are non-enzymatic binding assays, that typically include antigen-antibody pair systems, and which preferably provide chromatographic indications. The typical rapid chromatographic tests utilize either a "sandwich" assay or a "competition" assay to detect the presence of a desired analyte. In the sandwich assay, an analyte is bound, or "sandwiched," between an unlabeled first binding partner and a labeled second binding partner. For example, an analyte, such as a protein hormone, can be captured by a first binding partner, in this case, a first antibody immobilized on a membrane. The analyte-first antibody complex can then be detected by a second binding partner having a label, such as a second antibody tagged with a colored particle. In contrast, during the competition assay, the analyte in the sample competes with a labeled analyte, or labeled analogue to the analyte, for a binding partner immobilized on a solid support. A greater concentration of analyte in the sample results in a lower signal in the assay, as the labeled analytes are competed away from the binding partner on the solid support (i.e., the signal produced during a competition assay decreases as the concentration of analyte in the sample increases). Thus, the sandwich assay can provide a qualitative or quantitative assessment with great sensitivity, while the competition assay provides a quantitative measure of analyte concentration over a broad range with less sensitivity. In these dry reagent systems a preferred form is microparticulate labels, and in particular latex particle systems.

The present invention is useful in assays which use specific binding members. A specific binding partner or member, as used herein, is a member of a specific binding pair -- that is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific

binding members include antigens, antigen fragments, antibodies, and antibody fragments, both monoclonal and polyclonal, and complexes thereof, including those formed by recombinant DNA molecules. The term hapten, as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

Analyte, as used herein, is the substance to be detected which may be present in the test sample. The analyte can be any substance for which there exists a naturally occurring specific binding member (such as, an antibody), or for which a specific binding member can be prepared. Thus an analyte is a substance that can bind to one or more specific binding members in an assay. Analyte also includes any antigenic substances, haptens, antibodies, macromolecules, and combinations thereof. As a member of a specific binding pair, the analyte can be detected by means of naturally occurring specific binding partners (pairs) such as the use of intrinsic factor protein as a member of a specific binding pair for the determination of Vitamin B12, or the use of lectin as a member of a specific binding pair for the determination of a carbohydrate. The analyte can include a protein, a peptide, an amino acid, a hormone, a steroid, a vitamin, a drug including those administered for the therapeutic purposes as well as those administered for illicit purposes, a bacterium, a virus, and metabolites of or antibodies to any of the above substances. In particular, such analytes include, but are not intended to be limited to, ferritin; creatinine kinase MIB (CK-MB); digoxin; phenytoin; phenobarbital; carbamazepine; vancomycin; gentamicin, theophylline; valproic acid; quinidine; luteinizing hormone (LH); follicole stimulating hormone (FSH); estradiol, progesterone; IgE antibodies; vitamin B2 microglobulin; glycated hemoglobin (Gly. Hb); cortisol; digitoxin; N-acetylprocainamide (NAPA); procainamide; antibodies to rubella, such as rubella-IgG and rubella-IgM; antibodies to toxoplasmosis, such as toxoplasmosis IgG (Toxo-IgG) and toxoplasmosis IgM (Toxo-IgM); testosterone; salicylates; acetaminophen; hepatitis B core antigen, such as anti-hepatitis B core antigen IgG and IgM (Anti-HBC); human immune deficiency virus 1 and 2 (HIV 1 and 2); human T-cell leukemia virus 1 and 2 (HTLV); hepatitis Be antigen (HbeAg); antibodies to hepatitis Be antigen (Anti-Hbe); thyroid stimulating hormone (TSH); throxine (T4); total triiodothyronine (Total T3); free triiodothyronine (Free T3); carcinoembryoic antigen (CEA); and alpha fetal protein (AFP).

Drugs of abuse and referenced substances include, but are not intended to be limited to, amphetamine; methamphetamine; barbiturates such as amobarbital, secobarbital, pentobarbital, phenobarbital, and barbital; benzodiazepines such as librium and valium; cannabinoids such as hashish and marijuana; cocaine; fentanyl; LSD; methaqualone; opiates such as heroin, morphine, codeine, hydromorphone, hydrocodone, methadone, oxycodone, oxymorphone, and opium; phenylcyclidine; and propoxyphene. The details for the preparation of such antibodies and the suitability for use as specific binding members are well known to those skilled in the art.

The analyte-analog can be any substance which cross-reacts with the analyte-specific binding member, although it may do so to a greater or lesser extent than does the analyte itself. The analyte-analog can include a modified analyte as well as a fragmented or synthetic portion of the analyte molecule, so long as the analyte-analog has at least one epitope site in common with the analyte of interest. An example of an analyte-analog is a synthetic peptide sequence which duplicates at least one epitope of the whole-molecule analyte so that the analyte-analog can bind to an analyte-specific binding member.

The test sample can be derived from any biological source, such as a physiological fluid, including whole blood or whole blood components including red blood cells, white blood cells, platelets, serum and plasma; ascites; urine; sweat; milk; synovial fluid; mucous; peritoneal fluid; amniotic fluid; pericerebrospinal fluid; and other constituents of the body which may contain the analyte of interest. The test sample can be pretreated prior to use, such as preparing plasma from blood, diluting viscous fluids, or the like; methods of treatment can involve filtration, distillation, concentration, inactivation of interfering compounds, and the addition of reagents. Besides physiological fluids, other liquid samples can be used such as water, food products and the like for performance of environmental or food production assays.

This invention is described in the context of the tests discussed below and illustrated in Figs. 1 – 8. For example, in the A1c test kits used, the sample dilution or sample preparation solution contains ferricyanide in a buffered solution containing a zwitterionic

surfactant. The purpose of the dilution buffer solution is to dilute the whole blood sample and to lyse the red blood cells. The zwitterionic surfactant is the hemolytic agent that lyses the red blood cells. It has been found that adding a nonionic surfactant, forming a dilution solution composition containing two different surfactants, results in improved stability of the test kit and improved shelf life of the test kit. The amount of nonionic surfactant useful in this particular application of the invention can be from at least about 0.01% w/v (weight of surfactant per volume of initial solution) up to an amount that may interfere with the function of the hemolytic agent or the specific binding assay. In general, the amount of nonionic surfactant that will provide desired stability and shelf life improvement can be from about 0.01% to about 10% w/v, preferably from about 0.1% to about 7% w/v, more preferably from about 0.2% to about 5% w/v and most preferably from about 0.3% to about 4% w/v. As seen from the Test Examples below, in this particular test kit, levels of 0.5% and 1.0% w/v provide excellent stability and shelf life improvement.

In general application of this invention, one skilled in the art can determine what type of surfactant is used or is useful in the sample preparation solution, i.e., the surfactant that reacts with or converts the analyte to a form needed for the assay to be performed. Once the type of surfactant is determined, the selection of a different type surfactant to provide stability and shelf life enhancement will be apparent to one skilled in the art. As disclosed above, if the analyte modifying surfactant is a zwitterionic surfactant, then the second surfactant can be a nonionic and/or ionic surfactant, whichever provides the desired improved stability of the system. If the analyte modifying surfactant is a nonionic surfactant, then the second surfactant can be a zwitterionic and/or ionic surfactant, whichever provides the desired improved stability of the system. If the analyte modifying surfactant is an ionic surfactant, then the second surfactant can be a nonionic and/or zwitterionic surfactant, whichever provides the desired improved stability of the system. The amount of the first analyte modifying surfactant in a particular system will be determined by the analyte content in the sample to be tested. The amount of the second stabilizing surfactant will range from about 0.001% to about 15% w/v, about 0.01% to about 10% w/v, about 0.05% to about 8% w/v or about 0.1% to about 5% w/v. Formulations for a particular test kit and application can be

devised following the disclosure herein and by straightforward testing, as illustrated in the following Test Examples.

The selection of surfactants according to this invention will likewise be within the skill of the art following the disclosure herein. For example, in the test examples below, the stabilizing surfactant can be a compatible nonionic surfactant, such as the SURFYNOL® liquid nonionic surfactants (available from Air Products and Chemicals, Inc., Allentown, Pennsylvania), particularly the 400 Series, such as the 440, 465 and 485 products, which are ethoxylated acetylenic glycols. Other surfactants can be selected from the PLURONIC® and TETRONIC® lines of surfactants (available from BASF Performance Chemicals, Parsippany, New Jersey), particularly the "L Series" EO-PO-EO type or the "R Series" PO-EO-PO type. Other commercially available surfactants for formulation according to this invention will be known to one skilled in the art.

TEST EXAMPLES

This invention was tested using standard METRIKA® A1c test kits available from Metrika, Inc., Sunnyvale, California. The test kits, as supplied, include the Sample Dilution Buffer, which contains a hemolytic zwitterionic surfactant, which is Zwittergent 3-14 available from Roche Applied Science, Roche Diagnostics Corporation, Indianapolis, Indiana. Some of the test kits were used as supplied for comparison, and some of the test kits were modified by adding a nonionic surfactant to the Sample Dilution Buffer solution in the amounts shown in the following test results. The surfactant selected for the tests was SURFYNOL® 485 nonionic surfactant available from Air Products and Chemicals, Inc., Allentown, Pennsylvania.

The experimental results are shown in the Figures 1 - 8. The y-axis for Figs. 1 and 2 is "Zone 1 R change" which is the difference between reflectance values for Zone 1 (the HbA1c immunoassay zone) obtained from units stressed at 45°C versus 2-8°C. In these experiments, A1c units were stressed at 45°C and 2-8°C for 15 days. Stressing at 45°C is commonly used as an accelerated means of assessing product stability. The projected shelf

life at room temperature is typically a multiple of the shelf life at 45°C. The "units" referred to herein are the METRIKA® A1c monitor that provides a port for receiving the diluted blood sample, the reagent test zones built into the unit, and an LED readout for providing the user the results of the test. "STB" is the Sample Treatment Buffer, also referred to above as the Sample Dilution Buffer. "SOP STB" is the standard Sample Dilution Buffer as conventionally used in the A1c kits, without addition of the nonionic surfactant. The STB, itself, was not stressed for the tests shown in Figs. 1 and 2. As can be seen for both lots K-36 (Lot Number 0307213), Fig. 1, and K-41 (Lot Number A3-06-010), Fig. 2, addition of Surfynol® 485 to the STB restored unit performance to its original level prior to stress – when tested with a fresh whole blood sample and assuming no change in performance for the 2-8°C condition. This was true for a range of Surfynol® 485 concentrations from 0.5% to 3.0% w/v (weight of Surfynol® 485 per volume of SOP STB).

A more extensive and detailed study, Stability Protocol SP-03-006, was performed next (with K-50, Lot Number 0316004) to establish the impact of the two lowest Surfynol® 485 concentrations from the previous experiment on stability for up to four months. Lyphocheck A1c Controls from Bio-Rad Laboratories, Inc., Hercules, California, Levels 1 and 2,were used for testing at each time point to detect any potential change in accuracy for different regions of the assay's dynamic range. These results are shown in Figs. 3 - 8. The y-axis for these figures is "%A1c" which is the ratio of HbA1c concentration to total hemoglobin concentration, expressed as a percentage. The SOP buffer (STB without added surfactant) failed after 3 to 7 days at 45°C – as has been commonly observed in the past, with room temperature stability being compromised at about 3 months (Figs. 3 and 4). In contrast, 45°C stability was improved to 3-4 months when 0.5% - 1.0% Surfynol® 458 was added to the STB (Figures 5-8). Room temperature stability exceeded 4 months and, as a result of the approximately tenfold enhanced 45°C stability, is expected to remain stable for several-fold longer. Unlike the earlier study, in these tests both the units and the STB samples were stressed under the same conditions.

Other details of selection of surfactant combinations for use in this invention will be apparent to one skilled in the art following the disclosure herein. The scope of this invention is defined by the following claims.